AMENDMENTS TO THE SPECIFICATION

Please amend the Title as follows.

[FARNESYL] MUTANT PRENYL DIPHOSPHATE SYNTHASE

Please amend col. 1, lines 14-25, as follows.

Of the substances having important functions in organisms, many are biosynthesized using isoprene (2-methyl-1,3-butadiene) as a constituent [units] unit. These compounds are also called isoprenoids, terpenoids, or terpenes, and are classified depending on the number of carbon atoms into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), and the like. The actual biosynthesis starts with the mevalonate pathway through which mevalonic acid-5-diphosphate is synthesized, followed by the synthesis of isopentenyl diphosphate (IPP) which is an active isoprene unit.

Please amend col. 1, lines 39-50, as follows.

There are Z type and E type condensation reactions. Geranyl diphosphate is a product of E type condensation and neryl diphosphate is of Z type condensation. Although, the all-E type is considered to be the active form in farnesyl diphosphate and geranylgeranyl diphosphate, the Z type condensation reaction leads to the synthesis of natural rubber, dolichols, bactoprenols (undecaprenols), and [plants] various polyprenols found in plants. They are believed to undergo the condensation reaction using the phosphate ester bond energy of the pyrophosphate and the carbon backbone present in the molecule and to produce pyrophosphate as the byproduct of the reaction.

Please amend col. 1, line 66 – col. 2, line 12, as follows.

Furthermore, via the biosynthesis of these active-form isoprenoids, a vast number of kinds of compounds that are vital to life have been synthesized. Just to mention a few, there are cytokinins that are plant hormones and isopentenyl adenosine-modified tRNA that use hemiterpenes as their precursor of synthesis, [geraniols] geraniol and [that] its isomer, nerol, belonging to monoterpens that are the main components of rose oil perfume and a camphor tree extract, camphor, which is an insecticide. Sesquihormones include juvenile hormones of insects, diterpenes include a plant hormone gibberellin, trail pheromones of insects, and retinols and

retinals that function as the visual pigment precursors, binding components of the purple membrane proteins of highly halophilic archaea, and vitamin A.

Please amend col. 3, lines 41-59, as follows.

It has been found out that of the two aspartic acid-rich domains that have been proposed based on the amino acid sequence of the prenyl diphosphate synthase, the amino acid residue located at the fifth position in the N-terminal direction from the conserved sequence I [(DDXX(XX)D)] ($\underline{D_1D_2X_1(X_2X_3)X_4D_3}$) (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) of the aspartic acid-rich domain in the amino-terminal side is responsible for controlling the chain length of the reaction product. Hence, a method has been invented that controls the reaction product for the purpose of lengthening the chain length of the reaction product (Japanese patent application No. 8-191635 filed on Jul. 3, 1996 under the title of "A Mutant Prenyl Diphosphate Synthase"). The enzyme produced using the method enables production of reaction products that have several chain lengths. However, methods [have not been] are not known that [induce mutation] include mutations of geranylgeranyl diphosphate synthase to control the reaction products [to be in the short chain-length side] having a shorter chain length in order to produce farnesyl diphosphate.

Please amend col. 3, line 62 – col. 4, line 3, as follows.

It is an object of the invention to establish a process for producing farnesyl diphosphate synthases by modifying amino acid sequences of prenyl diphosphate enzymes. A new enzyme that is more stable or that has a high specific activity more adaptable to industrial application would make it possible to obtain immediately a mutant prenyl diphosphate synthase or the gene thereof that produces farnesyl diphosphate and that retains the property [owned] exhibited by [the] the prenyl diphosphate synthase prior to mutation.

Please amend col. 4, lines 4-14, as follows.

From the information on the nucleotide sequence of the gene of the geranylgeranyl diphosphate synthase of the mutant Sulfolobus acidocaldarius (S. acidocaldarius), it was clarified that out of the two Aspartic acid-rich domains that have been proposed based on the analysis of the amino acid sequence of prenyl diphosphate synthases, the amino acid residues within the

aspartic acid-rich domain conserved sequence I [(DDXX(XX)D)] ($D_1D_2X_1(X_2X_3)X_4D_3$) at the amino terminal side or the five amino acid residues to the N-terminal side from the amino terminal of said conserved sequence I are involved in the control of chain length of the reaction products.

Please amend col. 4, lines 15-34, as follows.

Thus, the present invention provides a mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain [(DDXX(XX)D)] ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located [at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain] one amino acid position downstream of D_2 has been substituted by another amino acid, and/or

additional amino acid(s) have been inserted in between the [amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal] first amino acid downstream of D_2 and the first amino acid upstream of D_3 of said aspartic acid-rich domain.

Please amend col. 5, lines 30-43, as follows.

It has been proposed that there are five conserved regions in the amino acid sequence of a prenyl diphosphate synthase (one subunit in the case of a heterodimer) (A. Chem et al., Protein Science Vol. 3, pp. 600-607, 1994). It is also known that of the five conserved regions, there is an aspartic acid-rich domain conserved sequence I [(DXX(XX)D)] $D_1D_2X_1(X_2X_3)X_4D_3$ (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) in region II. Although there is also an aspartic acid-rich domain indicated as "DDXXD" in region V, the aspartic acid-rich domain used to specify the modified region of the amino acid sequence

of the present invention is the one present in region II, and this domain is termed as the aspartic acid-rich domain I as compared to the aspartic acid-rich domain II present in region V.

Please amend col. 5, line 66 – col. 6, line 16, as follows.

In accordance with the present invention, in the amino acid sequence of a prenyl diphosphate synthase, at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain [(DDXX(XX)D)] $\underline{D_1D_2X_1(X_2X_3)X_4D_3}$ (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located [at the first position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain] <u>one amino acid position downstream of D_2</u> has been substituted by another amino acid, and/or

an additional amino acid(s) have been inserted in between the [amino acid residue located at the first position in the N-terminal side from D of the C-terminal and D of said C-terminal] <u>first amino acid downstream of D_2 and the first amino acid upstream of D_3</u> of said aspartic acid-rich domain.

Please amend col. 7, line 66 – col. 8, line 7, as follows.

It is known that the distance between the sequence of the ribosome [biding] <u>binding</u> site (GGAGG and similar sequences thereof) and the initiation codon ATG is important as the sequence regulating the ability of synthesizing protein from mRNA. It is also well known that a terminator (for example, a vector containing rrn PT_1 T_2 is commercially available from Pharmacia) that directs transcription termination at the 3'-end affects the efficiency of protein synthesis by a recombinant.

Please amend col. 9, lines 12-21, as follows.

By using the method of producing the mutant prenyl diphosphate synthase obtained by the present invention, the mutant prenyl diphosphate synthase derived from [a] <u>an</u> archaea may

be created that is more stable and thus easier to handle and that produces [prrenyl] prenyl diphosphate. Furthermore, there is also expected a creation of the farnesyl diphosphate-producing mutant prenyl diphosphate synthase that has the property of the prenyl diphosphate synthase prior to mutation (for example, salt stability or stability in a wide range of pH) added thereto.

Please amend col. 10, lines 3-4, as follows.

Construction of a Plasmid Containing the Gene for [Geranylaeranyl] Geranylgeranyl Diphosphate Synthase

Please amend col. 10, lines 52-62, as follows.

Introduction of the mutation (F77Y, T78S, V80I, I84L, 84PS85) was effected using two nucleotides. First, mutation was introduced as mentioned in Example 3 using the oligonucleotide

5'-GTTCTTCATACTTATTCGCTTATTCATGATAG [TATT-31]TATT-3' (SEQ ID No: 7) and a transformant was prepared in accordance with Example 4, and furthermore mutation was introduced into the plasmid thus obtained using the oligonucleotide

5'-ATTCATGATGATCTTCCATCGATGGATCAAGAT-3' (SEQ ID No: 8).

Please amend col. 11, lines 16-27, as follows.

These primer DNA's were subjected to phosphorylation treatment at 37° C. for 30 minutes in the reaction medium shown below followed by denaturation at 70° C. for 10 minutes:

10 pmol/μl primer DNA 2 μl

10×kination buffer 1 μl

10 mM ATP 1 μl

[H2O] H_2O μl

T4 polynucleotide kinase 1 μl

wherein the 10×kination buffer is 1000 mM Tris-Cl (pH 8.0), 100 mM $MgCl_2$, at 70 mM DTT.

Please amend col. 11, lines 47-58, as follows.

The single strand DNA thus obtained was used as the template in the reaction in which a primer DNA for synthesizing a complementary strand was treated in the following reaction solution at 65° C. for 15 minutes and then annealed by allowing to stand at 37° C. for 15 minutes:

Single strand DNA 0.6 pmol

Annealing buffer solution 1 μl

Primer DNA solution (Example 2) 1 μl

[H2O] H_2O make to a final volume of 10 μl

in which the annealing buffer solution is 200 mM Tris-Cl (pH 8.0), 100 mM $MgCl_2$, 500 MM NaCl, and 10 mM DTT.

Please amend col. 12, lines 16-42, as follows.

Of the transformants obtained as above, the substitution-mutated pBs-SacGGPS plasmid that has a cleavage site of BspHI, EcoRV or ClaI was selected. The nucleotide sequence in the neighborhood of the codon corresponding to the amino acid residue that undergoes mutation of the SacGGPS gene of the selected substitution-mutated pBs-SacGGPS plasmid was determined by the dideoxy metho. As a result, the pBs-SacGGPS plasmid containing the following five mutated SacGGPS genes was obtained. The nucleotide sequences encoding the amino acid sequences from the amino acid at position 77 to the amino acid at position 85 is shown below:

Mutation Nucleotide sequence

T77F, H81A: 5′–TTTTTCCTTGTGGCTGATGATATCATG-3′ (SEQ ID No: 9)

T78P, H81L: 5′–TTTTTCCTTGTGCTTGATGATATCATG-3′ (SEQ ID No: 10)

F77Y, T78F, H81L: 5′-TATTTCCTTGTGCTTGATGATATCATG-3′[31] (SEQ ID No: 11)

F77Y, T78F, H81A: 5′–TATTTCCTTGTGGCTGATGATATCATG-3′ (SEQ ID No: 12)

F77Y, T78S, V80I, I84L, 84PS85: 5′-TATTCGCTTATTCATGATGATCTTC CATCGATG-3′ (SEQ ID No: 13)

Wild type: 5′–TTTACGCTTGTGCATGATGATATTATG-3′ (SEQ ID No: 14).